Interfacial Protein-Lipid Interactions I

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Revealing Conformational Substates of Lipidated N-Ras Protein by Pressure Modulation

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Signaling networks maintain their spatiality by localization of their protein constituents to distinct regions of the membrane, and the different Ras guanine nucleotide binding proteins are a paradigm example of this. Despite being highly homologous, they exhibit isoform specific diversity in generating explicit signal outputs governed by, but not limited to, their hyper variable region responsible for targeting them to particular membrane microdomains (1). In addition, Ras proteins are known to sample multiple conformations which exhibit varying affinities towards their interaction partners. To fully explore the conformational space exhibited by Ras, experimental identification of conformational substates and characterization of conformational equilibria are mandatory. We applied pressure modulation in combination with FT-IR spectroscopy to reveal equilibria between spectroscopically resolved, otherwise low lying, substates of the lipidated signaling protein N-Ras in its different nucleotide binding states and in the absence and presence of a model biomembrane. Not only the nucleotide binding, but also the presence of the membrane has a drastic effect on the conformational dynamics and selection of conformational substates of the protein, and a new substate appearing upon membrane binding could be uncovered. Population of this new substate is accompanied by structural reorientations of the G-domain involving α-helix-membrane interactions. These findings thus illustrate that the membrane controls signaling conformations by acting as an effective interaction partner which has consequences for the G-domain orientation of membrane-associated N-Ras which in turn is known to be critical for its effector and modulator interactions. Finally, these results provide first insights into the influence of pressure on Ras-controlled signaling events in organisms living under extreme environmental conditions as they are encountered in the deep sea.

Reference

1. Weise K, Kapoor S, ... Waldmann H, Winter R (2011) *J Am Chem Soc* 133:880-887.

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Importance of Aromatic Anchor Residue Identity and Location for the Tilt and Dynamics of Transmembrane Peptides

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Aromatic or polar amino acid residues often flank each side of a membrane-spanning alpha helix, whether in an integral membrane protein or a model peptide. The aromatic residues Trp, Tyr and to a lesser extent Phe tend to partition to the membrane-water interface and act as âœanchorsâ to stabilize the transmembrane orientation. The synthetic model peptide, GWALP23 (acetyl-GGALW^5(LA)$_6$LW^{19}LAGA-[ethanol]amide), has proven valuable for experimentation, with only one Trp âœanchorâ near each end of the transmembrane sequence. Indeed, with relatively minimal complications from the peptide dynamics, the average tilt of GWALP23 has been shown to vary systematically in lipid bilayer membranes of different thickness (see J. Biol. Chem. 285, 31723). We have employed ^{2}H-alanines and solid-state NMR spectroscopy to investigate the consequences of moving or replacing W5 or W19 in GWALP23 with selected Tyr, Phe or Trp residues at the same or nearby locations. We find that GWALP23 peptides having Y5, F5 or W5 exhibit essentially the same average tilt in bilayer membranes of DOPC, DMPC or DLPC; with somewhat increased dynamics for the F5 peptide. When âœdoubleâ anchors are present in Y^{4,5}GWALP23 or F^{4,5}GWALP23, the peptides appear less responsive to the bilayer thickness, as the dynamics become dramatically more extensive. Moving W19 to position 18, a 100° radial change, alters the direction of the helix tilt, as expected. We conclude that, in the absence of other functional groups, the aromatic residues determine the preferred orientations and dynamics of transmembrane peptides. Increased dynamics are observed when the ring hydrogen bonding is removed (Phe), or when two aromatic anchors are present on one side of the core transmembrane sequence.

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Response of GWALP Transmembrane Peptides to Titration of a Buried Lysine

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Designed α-helical peptides such as GWALP23 serve as useful models for probing the influence of polar amino acids within a core transmembrane helical sequence. We incorporated lysine as a guest residue into the membrane-spanning host peptides GWALP23 and closely related Y^{5}GWALP23 (acetyl-GGAL(W^5/Y^5)LALALAL^{12}AL^{14}ALALW^{19}LAGA-amide). Lysine was introduced at either position 12 or 14 of the host sequences, for which position 12 corresponds to the center. Solid-state NMR spectra of ^{2}H-Ala residues in peptides incorporated into oriented lipid bilayer samples reveal that L14K mutant peptides adopt well-defined orientations in DOPC, DMPC and DLPC. In each lipid membrane, the L14K substitution increases the helix tilt at neutral pH. The L12K substitution, on the other hand, reduces the ^{2}H NMR spectral quality at neutral pH, particularly in the thicker DOPC, suggesting a lack of distinct orientation, as the system struggles to insert a charged lysine into the thicker bilayers. As the positively charged K12 amino group is titrated to higher pH values, nevertheless, the ^{2}H NMR spectral quality improves in DOPC, and the K12 peptides adopt an average orientation nearly matching the one found for both host peptides GWALP23 and Y^{5}GWALP23 (with L12). In similar fashion, titration of K14, in any of the tested lipid bilayer membranes, results in a smaller helix tilt, again much closer to that observed for the L14 peptides without a polar guest residue. Steady-state fluorescence measurements using the Y^{5}GWALP23 series of peptides reveal spectral narrowing and modest blue shifts in Δ_{max} from the W19 reporter, when either K12 or K14 is rendered non-ionized, suggesting a somewhat more hydrophobic environment for the Trp indole ring when the guest Lys side chain is neutral.

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Characterization of Antimicrobial Peptides Relating to Shortened RWALP Model Peptides

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In the face of increasing bacterial drug resistance, small membrane-active peptides offer potentially attractive avenues to alternative antimicrobial agents. The goals of this project are to characterize the lipid interactions and analyze the antimicrobial efficacy of model peptides that are longer than the lactoferrin-derived, surface-acting LfB6 (RRWQWR-NH$_2$), yet shorter than the transmembrane RWALP23 (acetyl-GRALW(LA)$_6$LW-LARA-NH$_2$). New-generation RWALP peptides, of general sequence (ac-GR$_n$W$_m$(LA)$_j$LW$_m$R$_n$A-NH$_2$) were designed with varying total lengths (13-15) and numbers of Arg and Trp residues. ^{2}H-alanines were incorporated at several positions to serve as probes for recording solid-state NMR spectra from mechanically aligned samples of the peptides in bilayers of DLPC, DMPC or DOPC. Labeled RWALP13 (j=3, n=1, m=1) exhibits partial water solubility, no antimicrobial activity, and ^{2}H-NMR spectra characteristic of isotropic motion, even in the presence of lipid bilayers. Circular dichroism spectra of RWALP13 suggest partial α-helical character in water that is enhanced when lipids are present. The ^{2}H-NMR spectra indicate that the longer 14- or 15-residue peptides are aligned to varying degrees in the different lipid bilayer membranes. Antimicrobial assays reveal that peptides with four arginines have higher activity than those with only two arginines. Interestingly, within the 4-Arg category, RRWALP15 (j=3, n=2, m=1) shows higher activity (MIC of 6.25 μ1/4g/ml) against E. coli than does RRWWALP15 (j=2, n=2, m=2; MIC of 25 μ1/4g/ml). The ^{2}H-NMR spectra of RRWALP15 suggest significant alignment of the peptide with respect to lipid bilayer membranes. The combined antimicrobial and spectral features make RRWALP15 an especially good candidate for further analysis.

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Membrane Interactions of an Acylated and Non-Acylated Lactoferricin Peptide by Solid-State NMR and Fluorescence Spectroscopy and Molecular Dynamics Simulations

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LfB6 (RRWQWR-NH$_2$) is a tryptophan- and arginine-rich cationic antimicrobial peptide, derived from bovine lactoferrin, with broad spectrum activity that can be enhanced by N-terminal acylation (CH$_3$(CH$_2$)$_4$CO-RRWQWR-NH$_2$; C6-LfB6). The arginines promote selective interaction with negative

charges present in bacterial cell membranes, while the tryptophans prefer the membrane-water interface. We have combined solid-state ^{2}H and ^{31}P NMR and fluorescence experiments with all-atom and coarse-grained molecular dynamics simulations to investigate the interactions of LfB6 and C6-LfB6 peptides in bilayers composed of two distinct compositions: 3:1 POPE:POPG (anionic) and POPC (zwitterionic). Previously we reported that the arginines of C6-LfB6 are first to associate with POPE:POPG; whereas in POPC the C6 tail associates first. Solid-state ^{2}H NMR experimental results confirmed that the lipid order parameters are not significantly changed when C6-LfB6 is bound to negatively-charged membranes, while a slight decrease in order is observed for zwitterionic membranes (Romo, et al. 2011. *Biochim. Biophys. Acta*. 1808(8):2019-30). We now compare these results with those for non-acylated LfB6 peptide. Solid-state ^{2}H NMR spectra of mechanically aligned samples reveal that the order of POPC and of POPG is reduced in the presence of 1 mol% LfB6. By contrast, POPE shows an increase in order and some line broadening. ^{31}P NMR spectra indicate little effect on the lipid head group for either peptide. The Trp fluorescence emission maxima are blue-shifted for LfB6 (340 nm) and C6-LfB6 (336 nm) in POPE:POPG, compared to POPC (353 nm LfB6 and 348 nm C6-LfB6), suggesting that the Trp residues are less water-exposed in the anionic lipid membranes and when the C6 acyl chain is present.

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Tryptophan Probes at the Apolipoprotein C-III and Membrane Interface

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Lipoproteins are lipid-protein complexes which facilitate lipid transport throughout the body. The proteins which bind lipids to form these complexes are called apolipoproteins. Since apolipoproteins play essential roles in lipid metabolism, their dysfunction is associated with numerous diseases. Therefore, it is vital to gain a detailed molecular understanding of their interactions with lipid. In this work, we investigate the site-specific membrane interactions of apolipoprotein C-III (apoC-III) by measuring the steady-state fluorescence and time-resolved anisotropy of single Trp containing variants (W54F/W65F (W42), W42F/W65F (W54), W42F/W54F (W65)) in the presence of sodium dodecyl sulfate (SDS) micelles and phospholipid vesicles (1:1 molar ratio of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphate (POPA) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC)). Upon addition of vesicles or SDS micelles, apoC-III undergoes a secondary structural change from disordered to α-helical as indicated by circular dichroism (CD) spectroscopy. Using CD and fluorescence data, apparent membrane partition constants were extracted. Though secondary structural formation is comparable amongst the Trp variants upon vesicle binding, distinct site-specific Trp environments are observed. The penetration depths of W42, W54, and W65 into the vesicle bilayer also were assessed by using lipids labeled with the heavy-atom quencher, bromine, at different positions in the lipid hydrocarbon acyl-chain.

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Characterization of the Dynamical Properties of Ras Proteins upon Insertion into Model Biomembrane Systems

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Proteins of the Ras superfamily are located at the inner leaflet of the plasma membrane and play an important role in cellular signal transduction processes, such as cell growth or differentiation. Since nanoclustering is discussed as an effective signaling mechanism, determination of the rotational and translational dynamics of membrane-associated Ras proteins may help revealing additional mechanistic information about protein clustering and interactions with effector proteins. In this study, different biophysical approaches are combined to investigate the partitioning behavior and dynamical properties of two Ras isoforms, namely N- and K-Ras, in unilamellar vesicles of varied lipid composition. As model biomembrane systems, pure fluid-like (liquid-disordered), neutral and anionic heterogeneous model raft membranes were used. Confocal laser scanning microscopy was applied to gain information on the partitioning of the Ras lipoproteins into the different model membrane systems. Fluorescence anisotropy and fluorescence correlation spectroscopy experiments were carried out to yield information on dynamical properties, such as the rotational correlation time and the translational diffusion coefficient, of the fully lipidated BODIPY-labeled Ras proteins. The results reveal a preferential incorporation of Ras into fluid-like liquid-disordered lipid domains, independent of the Ras isoform and GDP/GTP-loading [1]. Although the rotational mobility remains quite high upon membrane insertion, the translational dynamics is limited by the viscosity of the respective lipid system, in agreement with results obtained in *in vivo* studies. Depending on the nucleotide loading, no significant differences could be detected with respect to the dynamics upon membrane incorporation.

Reference

[1] Weise K, Kapoor S, Denter C, Nikolaus J, Opitz N, Koch S, Triola G, Herrmann A, Waldmann H, and Winter R (2011) *J. Am. Chem. Soc*. ***133***:880-887.

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Single Molecule Study of the Oligomerization of Alzheimer's Aβ40 and Aβ42 on the Surface of Phospholipid Membranes

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The amyloid-β (Aβ) peptides 40 and 42 are believed to feature in the synaptic dysfunction and neuronal loss associated with Alzheimer's disease. One proposed mechanism for the synaptic loss is via the interaction between Aβ and cell surface neurotransmitters and receptors, which subsequently affect the cell signaling. Another hypothesis is that formation of calcium-permeable channels by Aβ oligomers on the membrane directly disrupts calcium homeostasis and triggers cell death. Both mechanisms are likely to involve peptide-membrane interaction where the amphipathic cell membrane provides an extensive surface for amyloid interactions and is the primarily cellular structure that Aβ contacts with. Most of our knowledge comes from experiments done at high Aβ concentration (~μM, as compared with the nM peptide concentrations in vivo) where peptide-peptide interactions in solution might bias the real peptide-membrane interaction. Using single molecule total internal reflection fluorescence microscopy, we observe single Aβ oligomers diffusing on the membrane at physiological concentration (~4nM). Using single molecule photobleaching and fluorescence intensity to assess the oligomers' sizes, we track individual peptide species as they diffuse in the membrane. Our studies reveal a mixture of freely diffusing and membrane-immobilized oligomers and show that the membrane accelerates Aβ dimer formation. A comparison of the membrane-bound oligomer species created by Aβ40 and Aβ42 yields information on how the two additional residues on Aβ42 affect the peptide-peptide and peptide-membrane interactions. These studies help further our understanding of the role of peptide-membrane interactions in the formation and growth of the amyloid-β oligomers that may contribute to Alzheimer's pathology.

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Characterization of Membrane Interactions with Lactoferricin Peptides by Both All-Atom and Coarse-Grained Molecular Dynamics Simulations, Solid-State NMR, and Fluorescence Spectroscopy

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LfB6 (RRWQWR-NH2) is a small cationic antimicrobial peptide with broad spectrum effectiveness that is derived from bovine lactoferrin. The mechanism for interaction between the antimicrobial peptide and the bacterial cell membrane is hypothesized to depend on lipid composition. Bacterial membranes generally contain a significant fraction of negatively charged lipids in contrast with zwitterionic mammalian membranes. Previously, we characterized the interactions of an acylated LfB6 (C6-LfB6) with a model bacterial membrane (3:1 POPE:POPG) and a model mammalian membrane (POPC). We observed that for C6-LfB6, the Arg residues lead the interaction with the POPE:POPG membrane, while the C6 tail is first to associate with the POPC membrane. Here, we investigate the interactions of the non-acylated LfB6 peptide with the same model membranes, using over 9 μs of all-atom molecular dynamics as well as 24 μs of coarse grained simulations and we compare our results to solid-state 2H and 31P NMR, and fluorescence spectroscopy. Molecular dynamics simulations reveal that the LfB6 peptide backbone does not penetrate as deeply in the model membranes as C6-LfB6. Further, both the arginines and tryptophans of LfB6 associate with both model membranes at the same time and the tryptophans of LfB6 are more deeply buried in the model mammalian membrane than with the acylated peptide. There is evidence in the simulation of hydrogen bonding to water by the tryptophans in both acylated and non-acylated peptides in spite of the low local water density and the burial depth found in the simulations. The results also show subtle changes in the membranes' structure between the acylated and non-acylated peptides.